

-2-

3. The method of Claim 1, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

4. The method of Claim 1, wherein the alginate salt is prepared from an alginate source selected from *Macrocystis pyrifera* and *Laminaria hyperborea*.

5. The method of Claim 1, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

6. The method of Claim 1, wherein the calcium releasing compound is D-glucono- δ -lactone.

7. The method of Claim 1, wherein the source of calcium ions is calcium carbonate and the calcium releasing compound is D-glucono- δ -lactone, and wherein the molar ratio of the calcium carbonate to the D-glucono- δ -lactone is 0.5.

8. The method of Claim 1, further comprising the step of implanting the three-dimensional crosslinked hydrogel system.

9. The method of Claim 1, wherein the three-dimensional crosslinked hydrogel system has a thickness of between about 4 mm and about 8 mm, and a diameter of approximately 18 mm.

10. The method of Claim 1, wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio of 0.27.

11. A method for tissue engineering *in vitro*, the method comprising the steps of:

mixing cells, an alginate salt and a source of calcium ions to provide a mixture;

-3-

adding a calcium releasing compound to the mixture to provide a crosslinked hydrogel;

selectively controlling shrinking, swelling or maintaining of the crosslinked hydrogel by varying a calcium ion concentration of a medium into which the crosslinked hydrogel is introduced; and

culturing the crosslinked hydrogel in the medium to provide a three-dimensional crosslinked hydrogel/cell system for growing the cells *in vitro*.

12. The method of Claim 11, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

13. The method of Claim 11, wherein the alginate salt is prepared from an alginate source selected from *Macrocystis pyrifera* and *Laminaria hyperborea*.

14. The method of Claim 11, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

15. The method of Claim 11, wherein the calcium releasing compound is D-glucono- δ -lactone.

16. The method of Claim 11, wherein the source of calcium ions is calcium carbonate and the calcium releasing compound is D-glucono- δ -lactone, and wherein the molar ratio of the calcium carbonate to the D-glucono- δ -lactone is 0.5.

17. The method of Claim 11, further comprising the step of implanting the three-dimensional crosslinked hydrogel/cell system.

18. The method of Claim 11, wherein the three-dimensional crosslinked hydrogel/cell system has a thickness of between about 4 mm and about 8 mm, and a diameter of approximately 18 mm.

-4-

19. The method of Claim 11, wherein the three-dimensional crosslinked hydrogel/cell system has a calcium ion to carboxyl molar ratio of 0.27.

20. The method of Claim 11, wherein the cells are osteoblasts.

22. The method as defined in claim 1 wherein the hydrogel system swelled at calcium ion concentrations in the medium between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration in the medium of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations in the medium between about 0.0020 M and about 0.0030 M.

23. A method for preparing a three-dimensional hydrogel system, the method comprising the steps of:

adding a calcium-releasing compound to a mixture of at least one hydrophilic polymer comprising an alginate salt and a source of calcium cations to provide a three-dimensional crosslinked hydrogel system; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a medium into which the hydrogel system is introduced.

25. The method as defined in claim 23 wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

26. The method as defined in claim 23, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

27. The method as defined in claim 26 wherein the calcium releasing compound is D-glucono- δ -lactone.

-5-

28. The method as defined in claim 27 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono- δ -lactone is 0.5.

29. The method as defined in claim 23 wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

30. The method as defined in claim 29 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

✓
Please cancel claim 31 without prejudice.

32. The method as defined in claim 31 wherein the hydrogel system swelled at calcium ion concentrations between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations between about 0.0020 M and about 0.0030 M.

33. The method as defined in claim 23, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells in vitro.

34. A three-dimensional crosslinked hydrogel composition, consisting essentially of:

at least one hydrophilic polymer comprising an alginate salt;

a source of calcium cations;

a calcium-releasing compound, whereby a mixture of the at least one hydrophilic polymer, the source of calcium cations and the calcium-releasing compound forms the crosslinked hydrogel composition; and

a culture medium into which the hydrogel composition is introduced, the culture medium having a predetermined calcium ion concentration, wherein the

-6-

predetermined calcium ion concentration determines the shrinking, swelling or maintaining of the crosslinked hydrogel composition.

35. The composition as defined in claim 34, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate; wherein the source of calcium cations is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate; and wherein the calcium-releasing compound is D-glucono- δ -lactone.

36. The composition as defined in claim 35 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono- δ -lactone is 0.5.

37. The composition as defined in claim 35 wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

38. The composition as defined in claim 37 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

40. The composition as defined in claim 34 wherein when the predetermined calcium ion concentration is between about 0.0020 M and about 0.0030 M, the hydrogel composition remains substantially the same size.

41. The composition as defined in claim 45 wherein the cells are at least one of osteoblasts and cells which secrete a medically useful compound.

42. The method of claim 2 wherein the cells secrete a medically useful compound.

43. The method of claim 11 wherein the cells secrete a medically useful compound.

-7-

44. The method of claim 33 wherein the cells are at least one of osteoblasts and cells which secrete a medically useful compound.

45. The three-dimensional crosslinked hydrogel composition as defined in claim 34, further comprising cells incorporated into the hydrogel composition, thereby forming a hydrogel/cell system.

46. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein when the predetermined calcium ion concentration is between about 0.0005 M and about 0.0010 M, the hydrogel composition swelled.

47. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein when the predetermined calcium ion concentration is about 0.0040 M, the hydrogel composition shrank.

48. The method as defined in claim 1 wherein the three-dimensional crosslinked hydrogel system is structurally homogeneous.

49. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein the composition is structurally homogeneous.

50. The method as defined in claim 1 wherein the source of calcium ions is in powder form.

51. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein the source of calcium cations is in powder form.

52. (New) A method for preparing a three-dimensional hydrogel system, the method comprising the steps of:

adding a calcium-releasing compound to a mixture of at least one hydrophilic polymer comprising an alginate salt and a source of calcium cations to provide a three-dimensional crosslinked hydrogel system, wherein the calcium

-8-

releasing compound is D-glucono- δ -lactone, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate, and wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a medium into which the hydrogel system is introduced, wherein the hydrogel system swelled at calcium ion concentrations between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations between about 0.0020 M and about 0.0030 M;

wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

53. (New) The method as defined in claim 52 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono- δ -lactone is 0.5.

Ex
com

54. (New) The method as defined in claim 53 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

55. (New) The method as defined in claim 55, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells in vitro.